Reference: *Biol. Bull.* **179**: 183–185. (October, 1990)

Occurrence of Partial Nuclei in Eggs of the Sand Dollar, *Clypeaster japonicus*

MITSUKI YONEDA[1] AND SHIN-ICHI NEMOTO[2]

[1]*Department of Zoology, Faculty of Science, Kyoto University, Kyoto 606 and* [2]*Tateyama Marine Laboratory, Ochanomizu University, Tateyama, Chiba 294, Japan*

Abstract. Females of *Clypeaster japonicus* bearing eggs with multiple nuclei were occasionally found. DAPI (4'-6-diamidino-2-phenylindole) stained all these nuclei. The summed volume of the two nuclei in binucleate eggs was similar to the nuclear volume in mononucleate eggs from the same batch. On fertilization, two partial nuclei migrated to the center of the egg with a time-course similar to that taken by a single nucleus; they then participated in forming the zygote nucleus, which subsequently formed a single mitotic spindle. These multiple nuclei thus appear to function as genuine nuclei. Possibly they result from the failure of a single nucleus to form during oogenesis.

Introduction

While studying the migration of female nuclei toward the center of echinoderm eggs for syngamy, Hamaguchi and Hiramoto (1986) noticed the occasional occurrence of two female nuclei in single unfertilized eggs of the sand dollar, *Clypeaster japonicus*. On fertilization of such eggs, both nuclei came to the center of the egg and contributed to the formation of a single zygote nucleus, which later underwent the normal cycle of mitosis. Boveri (1918) was the first to observe such "Partialkerne," which occur in a Mediterranean species of the sea urchin, *Parechinus microtuberculatus*. Referring to Boveri's discovery, Chambers and Chambers (1961) called them "partial nuclei."

Working on the early development of *Clypeaster japonicus*, we found, among the sand dollars obtained around the coast near Tateyama Marine Laboratory at Tokyo Bay, a few females bearing eggs with multiple nuclei. Because adults of *Clypeaster* are not abundant at the Laboratory, we relied upon incidental occurrences of such females. This note summarizes our occasional observations of "partial nuclei" during three seasons.

Received 30 April 1990; accepted 20 July 1990.

Observations

Because the unfertilized eggs of *Clypeaster* are transparent, we can easily identify the female nucleus under an ordinary microscope as a large clear spot about 10 μm in diameter. At least 1 in 20 females shed some eggs with more than one clear spot, each apparently representing a nucleus. Hamaguchi and Hiramoto (pers. comm.) have noticed that the rate of occurrence of such females along the coast of Sagami Bay is similar.

Fixing the eggs with methanol-acetic acid (3:1), rinsing three times with phosphate-buffered saline (PBS), and staining them with 4'-6-diamidino-2-phenylindole (DAPI, Sigma, at 0.25 μg/ml in phosphate-buffered saline), we confirmed that these clear spots are genuine nuclei (Fig. 1). The occurrence of multiple nuclei in a batch of eggs from a single female was variable, but typically about 10% are binucleate and 1% are trinucleate. The highest proportion of multiple nuclei recorded thus far was 43%; of these, 37% were binucleate and 6% were trinucleate.

The size of each partial nucleus is not uniform. They are usually smaller than the size of single nuclei, but the summed volume of the two partial nuclei is equal to the volume of a sphere 9 to 10 μm in diameter (Fig. 2), which compares well with the diameter of a single nucleus in unfertilized eggs. The size of eggs bearing partial nuclei is similar to that of mononucleate eggs. Thus the binucleate eggs do not appear to arise by fusion of two eggs, each with a single nucleus. Rather, the partial nuclei seem to derive from the incomplete fusion of karyomeres in the telophase of the second meiosis, as Boveri (1918) had already suggested.

Some minutes after insemination, both partial nuclei start to migrate towards the center of the egg, where they eventually fuse (Fig. 3) to form a single zygote nucleus. Figure 4 illustrates this migration, which is measured as a decrease in the radial distance of the nuclei from the

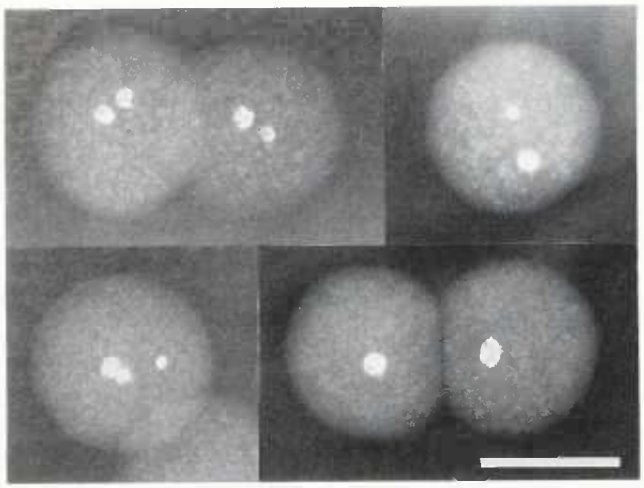

Figure 1. Unfertilized eggs stained with DAPI. Three eggs in the upper row are binucleates. One egg in the lower left is trinucleate. Two eggs (central and right in the lower row) are mononucleates. Scale bar = 100 μm.

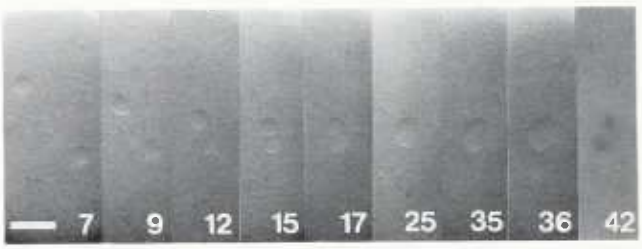

Figure 3. Fusion of partial pronuclei. Numerals are times in min after fertilization. The two pronuclei fused at 17 min. Sperm aster is not clear in this series of pictures. The picture taken at 36 min shows the nuclear envelope starting to break down. At 42 min, polarization-optical observation reveals the anaphase spindle. 26°C. Scale bar = 25 μm.

center of the egg. No difference was detected between the time course of a single nucleus and that of each of partial nuclei. According to Hamaguchi and Hiramoto (1986) two partial pronuclei fuse and then fuse with the sperm nucleus. Fused zygote nuclei formed a spindle (Fig. 3), and the time of the first cleavage was normal. Thus, partial nuclei can only be detected during a short interval of development, and they leave no noticeable trace of their occurrence.

Von Ledebur-Villeger (1972) and Mar (1980) found that the central migration of female nuclei still occurs, even in parthenogenetically activated sea urchin eggs without sperm asters. We confirmed this in *Clypeaster* eggs activated by treatment with 10 μg/ml Ca-ionophore (A 23187) for 2 min (Fig. 5), although the migration started later and was slower (Fig. 6) than that observed for normally fertilized eggs (also noticed by Mar, 1980). In any event, partial nuclei in activated eggs behaved similarly to single nuclei.

Microtubule inhibitors suppress the migration of nuclei towards syngamy (Zimmerman and Zimmerman, 1967; Schatten and Schatten, 1981; Hamaguchi and Hiramoto 1986). Movement of *Clypeaster* nuclei was stopped by treating eggs with 1 m*M* colchicine in seawater 5 min after activation. The movement of partial nuclei was similarly suppressed, and they remained separated, failing to form a single nucleus (Fig. 7). Yet both nuclei eventually exhibited the breakdown and reformation of their nuclear envelopes, as was observed in mononucleate eggs treated with either Colcemid (Sluder, 1979, 1986) or colchicine (Yoneda and Schroeder, 1984).

In several aspects, therefore, the partial nuclei behave very similarly to single nuclei.

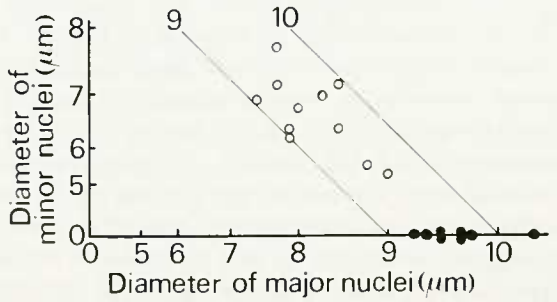

Figure 2. Diameters of partial nuclei measured in living unfertilized binucleate eggs. The diameters of smaller nuclei are plotted (open circles) on a cubic scale against the diameter of larger nuclei also on a cubic scale. Data are from a batch of eggs from a single female. The area between two diagonal lines ("9" and "10") indicates the domain within which the summed volumes of major and minor nuclei is equal to a sphere with a diameter between 9 μm and 10 μm. The diameters of single nuclei from the same batch were the control (filled circles).

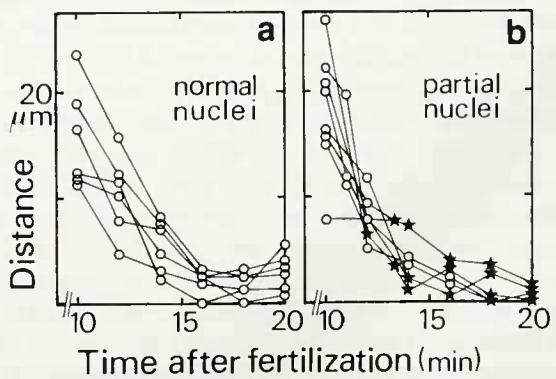

Figure 4. Migration of female pronuclei in normal (a) and binucleate (b) eggs after fertilization at 26°C. Migration indicated by the decrease in the distance (in ordinates) between pronuclei and the center of the eggs. Stars in (b) indicate fused nuclei.

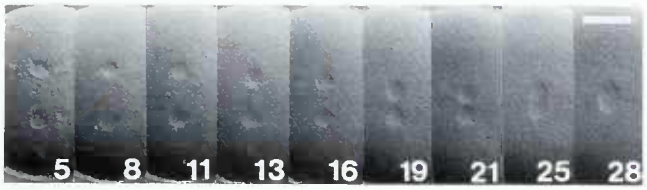

Figure 5. Migration and fusion of two partial nuclei on activation with Ca-ionophore. Times after activation are indicated by numerals. 26°C. Scale bar = 30 μm.

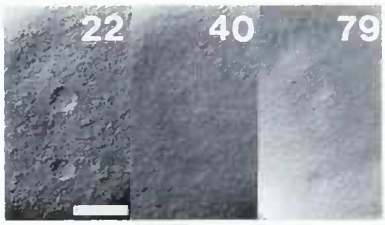

Figure 7. A pair of partial nuclei in activated eggs treated with 1 m*M* colchicine 5 min after activation. Numerals indicate the time after activation. Both nuclei remained separated, but their nuclear envelopes still broke down (40 min) and reformed (79 min). 26°C. Scale bar = 25 μm.

Remarks

Describing the presence of partial nuclei, Boveri's paper (1918) warns us of failure if we enucleate sea-urchin eggs by manual bisection, but overlook the occurrence of multiple nuclei. Thanks to the natural transparency of *Clypeaster* eggs, we can easily detect, with a low power microscope, batches of eggs including those with partial nuclei. Using eggs with partial nuclei may give us some insights into the process of nuclear migration and nuclear fusion in echinoderm eggs.

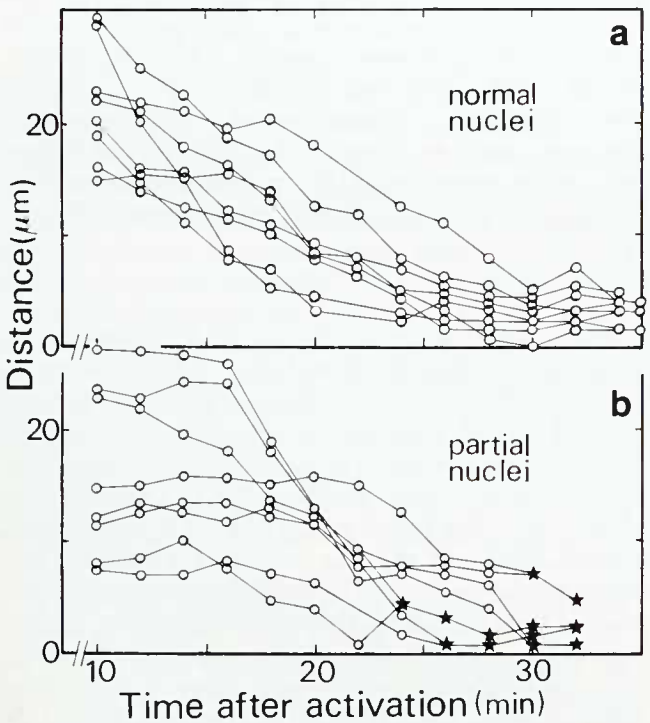

Figure 6. Migration of female pronuclei in normal (a) and binucleate (b) eggs on activation at 26°C, as indicated by decrease in the distance (ordinates) between pronuclei and the centers of the eggs. Stars in (b) mark fused nuclei. Data in Figures 4 and 6 are derived from a single batch. Note that the migration starts later and is slower than that observed in fertilized eggs (*cf.* Fig. 4).

Literature Cited

Boveri, T. 1918. Zwei Fehlerquellen bei merogonischen und die Entwicklungsfähigkeit merogonischer und partiell-merogonischer Seeigelbastarde. *Arch. Entwicklungsmech.* **44:** 419–471.

Chambers, R., and E. L. Chambers. 1961. *Explorations into the Nature of the Living Cell.* Harvard University Press, Cambridge.

Hamaguchi, M., and Y. Hiramoto. 1986. Analysis of the role of astral rays in pronuclear migration in sand dollar eggs by the Colcemid-UV methods. *Dev. Growth Differ.* **28:** 143–156.

Mar, H. 1980. Radial cortical fibers and pronuclear migration in fertilized and artificially activated eggs of *Lytechinus pictus*. *Dev. Biol.* **78:** 1–13.

Schatten, G., and H. Schatten. 1981. Effects of motility inhibitors during sea urchin fertilization. *Exp. Cell Res.* **135:** 311–330.

Sluder, G. 1979. Role of spindle microtubules in the control of cell cycle timing. *J. Cell Biol.* **80:** 674–691.

Sluder, G. 1986. The role of spindle microtubules in the timing of the cell cycle in echinoderm eggs. *J. Exp. Zool.* **238:** 325–336.

Von Ledebur-Villeger, M. 1972. Cytology and nucleic acid synthesis of parthenogenetically activated sea urchin eggs. *Exp. Cell Res.* **72:** 285–308.

Yoneda M., and T. H. Schroeder. 1984. Cell cycle timing in colchicine-treated sea urchin eggs: persistent coordination between the nuclear cycles and the rhythm of cortical stiffness. *J. Exp. Zool.* **231:** 367–378.

Zimmerman, A. M., and S. Zimmerman. 1967. Action of Colcemid in sea urchin eggs. *J. Cell Biol.* **34:** 483–488.